15.6.2015

MONDAY, 6/15

Petra & Tamannae

Checked which transformants contain right plasmids from the gel picture (ran on friday).

Table1

Plasmid	Correct colonies
AH011	4, 3 ,2
AH013	5
AH014	none
AH015	2

According to the table we didn't get any plasmid with medium RBS, so today we will transform only the new blue color producing plasmids (AH011) with strong (AH013) and weak (AH015) RBS.

Restriction Digestion

Checked NEB double digest finder to find right buffers for the restriction reactions

Table2

Restriction reaction	Buffer	Incubation temperature	Plasmid
EcoRI & PstI	NEBuffer 3.1	37 °C	AH009
EcoRI & SpeI	Restriction not recommended. Chose NEBuffer 3.1	37 °C	AH011
XbaI & pstI	CutSmart Buffer	37 °C	AH013 & AH015

Restriction reactions
Restricted all the colonies with the right plasmids.
Total reaction volume: 25 ul

Table3

Plasmid	Colony number	DNA added (ul)	Water added (ul)
AH009 (EcoRI & PstI)	From stock	4,9	16,6
AH011 (EcoRI & SpeI)	2	4,2	17,3
AH011 (EcoRI & SpeI)	3	2,8	18,7
AH011 (EcoRI & SpeI)	4	3,1	18,4
AH013 (XbaI & PstI)	5	5,2	16,8
AH015 (XbaI & PstI)	2	5,1	16,1

Added 2,5 ul correct buffer into each tube and 0,5 ul each enzyme (2 enzymes/reaction). Added enzymes last.
Incubated in 45 min 37°C to make sure that even the weaker reaction (AH011, EcoRI & SpeI) proceeds correctly. Followed the protocol.

Ligations
We did ligations of all the correct AH011 colonies (col. 2,3 & 4) to form 3 different AH016 and AH018 plasmids:

1. AH009 + AH011 col.2 + AH013 -> AH016
2. AH009 + AH011 col.3 + AH013 -> AH016
3. AH009 + AH011 col.4 + AH013 -> AH016
4. AH009 + AH011 col.2 + AH015 -> AH018
5. AH009 + AH011 col.3 + AH015 -> AH018
6. AH009 + AH011 col.4 + AH015 -> AH018

Ligation mix
2,7 ul each restricted DNA
1 ul T4 Buffer
0,5 ul ligase
0,4 ul water
Followed the protocol. Ligation incubation was longer than 30min, 71 min in total.

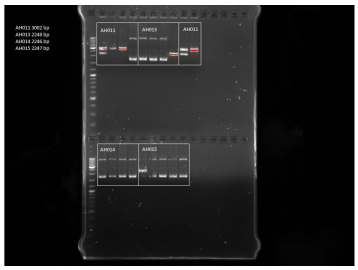
Transformation
Transformed ligations to TOP10 and BL21 -> 12 transformations in total

Plated transformants to chloramphenicol plates, one 200ul plate per transformation.

Picked four colonies from AH014 plate to do o/n cultures since none of the colonies screened on friday contained the right plasmid.

- colonies 6, 7, 8 and 9
- 2 ml LB with Kanamycin (0,8 µl)

Geldoc_2015-06-12_18hr_04min_-_EDITED.jpg



16.6.2015

TUESDAY, 6/16

Petra & Tamannae

Checked yesterday's transformations.
All the colonies were expected to be blue. Resulted that TOP10 colonies were both purple and colorless. All the BL21 colonies were colorless. Figured out that TOP10 cells can't read T7 promoter and can't produce blue color anyway. There was growth on each plate made yesterday.
We ought that AH009 backbone we used still contains the red color producing insert, and decided to create AH016, AH018 (and AH017) again using AH009 linearized backbone that doesn't contain the insert.

Transformation efficiency of BL21 cells is clearly lower than the efficiency of TOP10, since all the BL21 colonies contained only a couple of colonies.

Minipreps
Miniprepped o/n cultures of AH014 using GeneJET plasmid miniprep kit. Followed the protocol.

Restriction
Restricted AH009 (linearized), AH011 colony 4, AH013, AH014 (From the colonies 6, 7, 8, 9) and AH015 according to the table below.
Used AH014 from 4 different colonies since we don't know which colonies contain the right plasmid.
Also restricted these AH014 colony plasmids and ran them on gel to find if any of them were the correct thing.

Geldoc_2015-06-16_16hr_46min.jpg

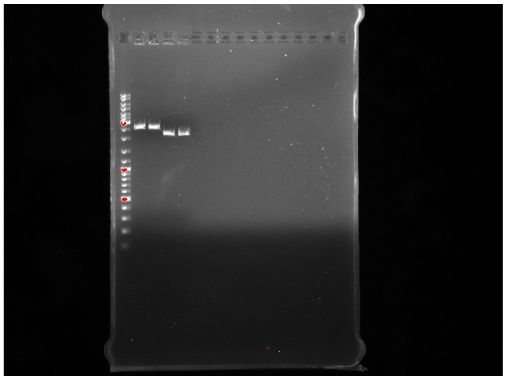


Table2

Restriction reaction	Buffer	Incubation temperature	Plasmid
EcoRI & PstI	NEBuffer 3.1	37 °C	AH009
EcoRI & SpeI	Restriction not recommended. Chose NEBuffer 1	37 °C	AH011
XbaI & PstI	CutSmart Buffer	37 °C	AH013, AH014, AH015

Restriction reactions
Total reaction volume: 25 ul

Table3

Plasmid	Colony number	DNA added (ul)	Water added (ul)
AH009	Linearized backbone	10,0	11,5
AH011	4	3,1	18,4
AH013	5	5,2	16,8
AH014	6	3,4	18,1
AH014	7	3,5	18,0
AH014	8	3,8	17,7
AH014	9	2,9	18,6
AH015	2	5,1	16,1

Added 2,5 ul correct buffer into each tube and 0,5 ul each enzyme (2 enzymes/reaction). Added enzymes last.
Incubated in 45 min 37°C to make sure that even the weaker reaction (AH011, EcoRI & SpeI) proceeds correctly. Followed the protocol.

Ligation:
AH016 and AH018 plasmids:

1. AH009 + AH011 + AH013 -> AH016
2. AH009 + AH011 + AH014 col. 6 -> AH017
3. AH009 + AH011 + AH014 col. 7 -> AH017
4. AH009 + AH011 + AH014 col. 8 -> AH017
5. AH009 + AH011 + AH014 col. 9 -> AH017
6. AH009 + AH011 + AH015 -> AH018

Ligation mix for AH018
There was enough NEB T4 DNA ligase for one ligation.
2,7 ul each restricted DNA
1 ul T4 Buffer
0,5 ul ligase
0,4 ul water
Followed the protocol. Ligation incubation was a bit longer than 30min.

We ran out of NEB T4 DNA ligase and used Amersham Biosciences Ready-To-Go T4 DNA Ligase for the rest of the ligations.

Ligation mix for AH016 and AH017
2,7 ul each restricted DNA
1 ul T4 Buffer
0,4 ul water
All the ingredients listed above + 10,6 ul water (total reaction volume 20,1 ul)
Followed Amersham Biosciences Ready-To-Go T4 DNA Ligase protool with these transformations.

Also did o/n cultures in 2 ml LB with appropriate antibiotic (Amp 1 ul, Kan 0,8 ul or Chl 1,5 ul) of AH007, AH008 and AH009, and AH016 and AH018 which were transformed to BL21(DE3). We chose several colonies.

17.6.2015

WEDNESDAY, 6/17

Anna in the lab

The o/n cultures had not been in shaking, the growth is very low. Minipreps only for BL21 o/n cultures. New o/n colonies of the backbones needed.

Chloramphenicol had been left o/n in an icebox. The ice had melted, but was still cold. I put the chloramphenicol back to the freezer.

Followed the MiniPrep protocol (Thermo Scientific GeneJET Plasmid MiniPrep Kit).
Because of the low amount of cells, the final elution was done in 25ul

Naming:
AH016 col 2 #1 -> AH016 2-1
AH016 col2 #2 -> AH016 2-2
AH016 col3 #1 -> AH016 3-1 etc.

Table1

Sample	Concentration (ng/ul)	260/280
AH016 2-1	31,9	1,93
AH016 2-2	30,8	1,94
AH016 3-1	39,4	1,91
AH016 3-2	25,3	1,92
AH016 4-1	54,1	1,78
AH016 4-2	44,7	1,98
AH018 2-1	36,1	1,98
AH018 2-2	46,0	1,98
AH018 3-1	45,1	1,94
AH018 3-2	42,1	1,98
AH018 4-1	55,1	1,89
AH018 4-1	37,5	1,97

Restriction digestion for all samples with EcoRI according to Restriction Digestion protocol.
Use 5ul DNA + 17ul H2O for AH016 4-1, AH018 2-2, 4-1 and 3-1
Use 6,5ul DNA + 15,5ul H2O for AH016 31-, 4-2, AH018 2-1, 3-2 and 4-2
Use 8ul DNA + 14ul H2O for AH016 2-1, 2-2 and 3-2
-> 30min +37C
-> 20 min + 80C
-> -20C

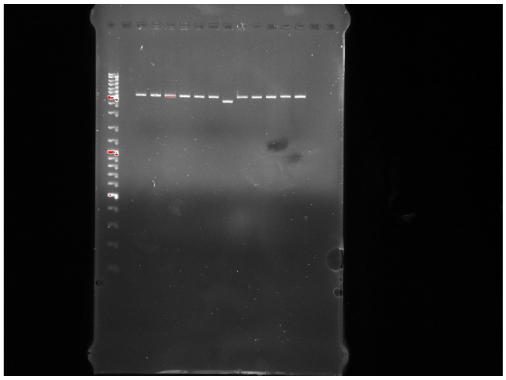
New o/n cultures of backbones AH007, AH008 and AH009
-2ml LB + 1ul amp for AH007
-2ml LB + 0,8ul km for AH008
-2ml LB + 1,5ul Chlor for AH009

Prepared a 1,2% agarose gel (50ml buffer + 0,65g agarose)

- prepared restricted plasmids for gel:
  - 10ul restriction mix + 2ul 6xLD
  - 100V, 40min
  - Wells:
    - 1 = AH016 2-1
    - 2 = AH016 2-2
    - ...
    - 12 = AH018 4-2

Transformed AH016, AH017 and AH018 (ligated yesterday) into BL21 competent cells
-> plated 200ul of each on chlor plates

Geldoc_2015-06-17_16hr_20min.png



18.6.2015

THURSDAY, 6/18

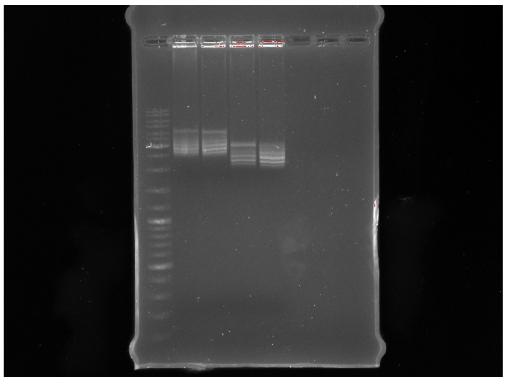
Stored yesterday's BL21-transformation plates to fridge after o/n incubation. Transformations were successful but colonies were not blue.

Restricted AH014 (colonies 6, 7, 8 and 9) with EcoRI following the protocol.

Ran restricted plasmids (AH014) in a gel (100 V, 1h). The gel consists of 50 ml 1 x TAE buffer, about 0,63 g agarose and 1,0 µl SYBR safe reagent. The pipeting order in the gel was:

1. ladder 3 µl
2. AH014 col 6 30 µl
3. AH014 col 7 30 µl
4. AH014 col 8 30 µl
5. AH014 col 9 30 µl

Geldoc_2015-06-18_14hr_08min.jpg



Did minipreps of AH007, AH008 and AH009 from the o/n cultures following mostly the protocol. However, the lysis reaction was ended with neutralization in about 20 min. Only AH007 col 1 & 2 were done in the time constraint (5 min).

NanoDrop results of the minipreps are in Table 1.

Table1

Sample	DNA (ng/µl)	Absorbance (A260/A280)
AH007 1	89,5	1,86
AH007 2	85,3	1,87
AH007 3	47,7	1,87
AH008 1	112,9	1,87
AH008 2	135,4	1,86
AH008 3	99,6	1,87
AH009 1	72,8	1,87
AH009 2	90,4	1,87
AH009 3	84,6	1,87

Restricted plasmid backbones (AH007, AH008 and AH009) with EcoRI and PstI to remove the insert. Doubled the protocol. Made three parallel reactions for AH007-1, AH008-2 and AH009-2, because they had the best DNA concentrations.

- 6µl AH007 + 37µl H2O
- 4µl AH008 + 39µl H2O
- 6µl AH009 + 37µl H2O

-> 30min +37C
-> 20min +80C

Ran restricted backbones on 1,2% agarose gel, 120V, 40min
-> gel purification kit
-> final elution in 25ul
-> stored in -20C
-> concentration not measured

Geldoc_2015-06-18_16hr_40min.jpg